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NASAL METHICILLIN- RESISTANT *STAPHYLOCOCCUS AURERUS* CARRIAGE IN EAST MALAYSIAN CHILDREN

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a community strain in the past decade. From October 2012 to March 2013, a total of 947 school children from eightprimary schools in the three districts of east Malaysia, ages between 7 years to 12 years, were screened for nasal carriage of MRSA. The overall prevalence of nares carriage of MRSA and *Staphylococcus aureus (*MSSA*)* were 3.4 % and 39% respectively. The carriage rate of MRSA was higher for children living in rural area (4.1%) than in the urban area (2.3%),aged 7-9 years (4.3%) than aged 10-12 years (2.7%) and Bajau ethnicity (4.!%) than other ethnicity. All 397 isolates were susceptible to vancomycin, ciprofloxacin, and gentamicin .MRSA isolates also showed susceptibility to rifampicin and cefuroxime. Of the 17 MRSA isolates available for molecular analysis, three clones with one major clone were identified and included sequence type (ST) 30- Staphylococcal chromosomal cassette (SCC*mec*) IV- Panton-Valentine leukocidin (PVL) genes-positive for 10 isolates, ST239-SCC*mec* III-PVL-negative-spa type t037 for 2 isolates and ST45-SCC*mec* IV-PVL-negative-t1081 for 5 isolates. Our study is population based and different from hospital based studies School children were screened from eight schools; therefore the prevalence of MRSA in the children may not reflect the entire population of Sabah, but MRSA nasal carriage may spread in the community. Future researchers have to determine several factors of nasal colonization and the efforts must be made to detect and prevent the spread of MRSA in the population

KEYWORDS: MRSA Nasal Carriage, School Children, Rural and Urban Area

INTRODUCTION

Community –associated methicillin –resistant *Staphylococcus aureus*(CA-MRSA) has become a global phenomenon [1]The CA-MRSA isolates were initially identified in pediatric populations and subsequently reported in certain adult populations, such as Native Americans, military recruits, prison inmates, drug users, men who have sex with men, and competitive sports participants [2,3,4]. Although superficial skin and soft tissue infections remained the most common manifestation of CA-MRSA, severe diseases such as necrotizing pneumonitis, necrotizing fasciitis, osteomyelitis, pyomyositis, septic embolism and venous thrombosis were not uncommon and previously caused death in healthy children [5,6]. The cause of the increasing incidence of CA-MRSA infection in previously healthy hosts are not completely understood, the factors influencing CA-MRSA virulence remain an issue of ongoing debate [7,8,9]. Colonization with *S.aureus* has been identified as an important risk factor for the development of S. *aureus*infections both in the community and hospital settings [10, 11]. Evidence further suggests that compared to methicillin-susceptible (MSSA), colonization with MRSA imposes a significantly greater risk for the development of subsequent infections [12]. In the past few years, the prevalence of MRSA colonization increased significantly among healthy hosts during CA-MRSA epidemics

[13, 14]. The carriage of MRSA with CA genotype was significantly higher among children than among adults [15]. CA-MRSA strains were recognized as novel pathogen that is genetically different from healthcare-associated MRSA, and five major epidemic clones have been identified worldwide. InTaiwan, a significantly increasing rate of MRSA carriage and infection amongst healthy subjects was observed in the past decade [16].Study was conducted to investigate the prevalence of MRSA colonization in healthy school children; survey covered three Sabah, districts. MRSA isolates were analyzed by molecular methods.

MATERIALS AND METHODS

Study Design-Setting

A retrospective study was conducted to determine the prevalence of MRSA nasal colonization in primary school children from the urban and rural areas of Sabah, Malaysia.Study included the national primary schools in the three districts e.g. KotaKinabalu, Papar and TuaranKota Kinabalu is categorized as the urban area, while Papar and Tuaran are categorized as rural area.

Ethical Clearance and Informed Consent

Prior permission from Malaysian Ministry of Education, Director of Education Sabah, and ethical clearance was obtained. Parents or guardian were provided with full information on the research project and informed consent of parent or guardian was obtained prior to nasal specimen collection. Subjects on active antibiotic therapy and past two week hospitalization were excluded

Nasal Specimen Collection

Primary school children age 7 to 12 years was involved in the study. A demographic detail of subjects was recorded in the study protocol. After all details completion nasal swabs were collected with Beckon Dickinson culture swabs with Stuart media (BD220093).

Microbiological Identification

All specimens were processed within three hours of collection. Columbia blood agar was used as primary culture, mannitol salt agar(BD), DNase test agar(BD), rapid test latex agglutination test for presumptive coagulase test, Avipath Staph, ODo44 kit(Omega Diagnostic, UK), and Gram staining was used for identification. Antibiotic resistance profile of *Staphylococcus aureus*isolates, determined by Bauer- Kirby disc diffusion techniques (1966) on Muller Hinton agar (BD), according to CSLI (formerly NCCLS) guidelines. Antibiotic assay included tests on sensitive control organism with batch of test strain *Staphylococcus aureus* ATCC 25923.Confirmation of methicillin resistance in *Staphylococcudaureus*-resistance to methicillin was examined by the method described by Barber (1964). MRSA isolates were tested for methicillin resistant determinant (*mec*A), product, penicillin binding protein 2 (PBP2) with rapid slide latex agglutination test by commercial available test kit (Denka Seiken, Japan).

Molecular Characterization

The extraction and purification of MRSA chromosomal DNA were conducted by using DNA kit (QIAamp® DNA Blood Mini Kit, QIAGEN). Pulsed-field gel electrophoresis (PFGE) with *Sma*I digestion was used to fingerprint all MRSA isolates according to the procedure described previously [17, 18]. The genotypes were designated in alphabetical order. PFGE patterns with fewer than 4-band differences from an existing genotype were defined as subtypes

of that genotype. Staphylococcal chromosome cassette *mec* (SCC*mec*) type and the presence of Panton-Valentine leukocidin (PVL) genes were determined by PCR assays according to the procedure described previously [17-21]. Multilocus sequence typing (MLST) [22] and *spa* typing [23] were performed for strains of representative PFGE patterns as described elsewhere

Statistical Analysis

Statistical Package for Social Sciences (SPSS) 17.0 software was used for analysis. Data analysis, odd ratios (ORs) was calculated to access the risk for each demographic factor. The significance of each odd ratios was then tested by Fisher-Extract Test .The differences between values were considered significant when $P<0.05$ was obtained.

RESULTS

In total, number of 1000 subjects were recruited from eight schools and included, Government primary school Tanjungaru ,Government Primary School Sri Gaya, Government primary School, Sri Mutiara, Government primary School, in Kota Kinabalu) Big Pangalat, Papar, Government Primary School, Suriati, in Papar and St. Philip primary school Tuaran and Government primary School Kiuluin Tuaran, In the study 947 nasal samples were obtained and examined for the presence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA). Fifty three subjects were excluded for incomplete information. Of 947, (63%) were rural subjects and 353 (37%) were urban subjects. The overall prevalence of nares carriage of MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA) were 3.4% and 39% respectively. In the rural areas of total 593 subjects were enrolled in the study, 235 (39.6%) were colonized with *Staphylococcus.* Of 235 isolates 211(36%) were methicillin susceptible *Staphylococcus aureus* (MSSA).of 235 isolates 24(75%) demonstrated to be CA-MRSA, Table 1. The carriage of MSSA, and CA-MRSA in the 7 to 9 years old were 47 %, (P= 0.797), and 58% (P= 0.097) respectively .The carriage in the 10 to years old, 53% (P= 0.797) and 42 % (P = 0.097) respectively Table 1.

In the urban areas of total 353 subjects were enrolled in the study, 162 (46. %) were colonized with *Staphylococcus*. Of 162 isolates 154(44%) were MSSA. 0f 162 isolates eight (25%) demonstrated to be CA-MRSA. The carriage of MSSA, and CA-MRSA in the 7 to 9 years old were 25% (P=0.006) and 37.5% (P=0.406). The carriage in the 10 to 12 years old 75% (P=0.006) and 62.5% (P=0.406), Table 1. Only age factor shows significant effect on the odds of MSSA. The age group of 10 to 12 years old shows significant odd ratio of MSSA to the age group 7 to 9 years old (P=0.006 <0.005). The MSSA, and CA-MRSA, nasal colonization in ethnic Bajau was higher in the rural areas were 58%, (P=0.664)100% and 79% (P=1.823) respectively, and in urban areas 43.5%(P=0.153) and 12.5% (P=0.410) Table 1. MSSA and, CA-MRSA nasal colonization was significantly higher in subjects ages > 7 to 9 years in the rural areas (47% and 58% respectively). MSSA and CA-MRSA nasal colonization was higher in subjects ages 10 to 12 years in the urban areas Table 1.

The detailed susceptibility distribution of various antimicrobials for the isolates is shown in the Table 2. All 397 *Staphylococcus* isolates were susceptible to vancomycin while moderate to high resistance to ampicillin, penicillin and erythromycin.

Seventeen MRSA isolates were available for molecular analysis and the detailed molecular characteristics are shown in Figure 1. Three PFGE patterns were identified, and included type AG with two subtypes for 10 isolates (59%), type BM for 5 (29%) and type A for 2. All 10 isolates of PFGE type AG carried type IV SCC*mec* and PVL genes and

belonged to ST30 but different spa types. The other two clones were characterized as PFGE BM-SCC*mec* IV-ST30-spa t1081-PVL-negative and PFGE A-SCC*mec* III-ST239-spa t037-PVL-negative, respectively.

Table 1: **Distribution of the Demographics and Nasal *Staphylococcus Aureus,* Carriage among 947 School Children in East Malaysia**

Variables	Total			Rural			Urban		
	Subject No.	MSSA No. (%)	MRSA No. (%)	Subject No. (%)	MSSA No. (%)	MRSA No. (%)	Subject No. (%)	MSSA No. (%)	MRSA No. (%)
Total (subtotal)	947	365 (39)	32 (3.4)	594	211 (36)	24 (4.1)	353	154 (44)	8 (2.3)
Male gender	551	213 (39)	19 (3.5)	304	112 (37)	11 (3.6)	247	101 (41)	8 (3.2)
Female gender	396	152 (38)	13 (3.3)	290	99 (34)	13 (4.5)	106	53 (50)	____
Age (Yrs)									
7-9	395	139 (35)	17 (4.3)	277	100 (36)	14 (5.1)	118	39 (33)	3 (2.5)
10-12	552	226 (41)	15 (2.7)	317	111 (35)	10 (3.2)	235	115 (49)	5 (2.1)
Ethnicity									
Bajau	491	189 (42)	20 (4.1)	351	122 (35)	19 (5.4)	139	67 (48)	1
Malay	212	83 (39)	4 (1.9)	108	37 (34)	2 (1.9)	104	46 (44)	2
Kadazan	203	79 (39)	7 (3.4)	118	46 (39)	3 (2.5)	85	33 (39)	4
Brunei	12	5 (42)	_____	6	3 (50)	_____	6	2 (33)	_____
Chinese	24	7 (29)	_____	10	3 (30)	_____	14	4 (29)	_____
India	5	2 (40)	1 (20)	_____	_____	_____	5	2 (40)	1

Table 2: Antimicrobial Susceptibility Pattern of Pathogen Isolated in Rural and Urban Schools

Areas	No. Isolates	Antimicrobial Susceptibility %								
		P	AM	E	GN	CXM	CAZ	CIP	RA	VA
Rural										
MRSA	24	72	67	67	94.5	94.5	67	100	100	100
MSSA	211	52	52	89	99.5	99.5	61	100	100	100
Urban										
MRSA	8	17	50	83.5	100	67	83.5	83.5	83.5	100
MSSA	154	17	45.5	91.5	100	99.5	84.5	100	100	100

Abbreviation: penicillin (P) ampicillin (AM), erythromycin (E), gentamicin (GN), cefuroxime(CXM), ceftazidime (CAZ), ciprofloxacine (CIP), rifampicin (RA), vancomycin (VA).

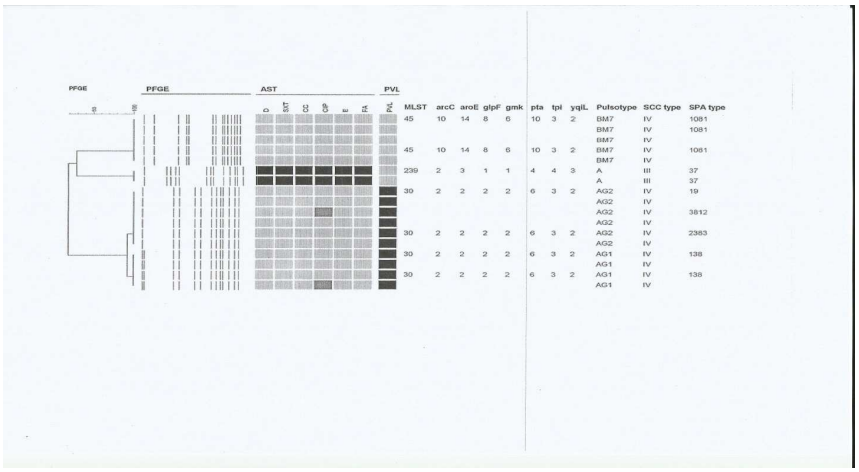

Figure 1: Distribution of Pulsed-Field Gel Electrophoresis (PFGE) Patterns and Other Molecular Characterization of 17 Methicillin-Resistant *S. Aureus* Isolate

Figure 1 molecular characterization of the 17 MRSA isolates. All isolates were susceptible to vancomycin, teicoplanin, andlinezolid, and resistant to penicillin. Antimicrobial susceptibility tests (AST): black indicates resistance, dark grey indicates intermediate resistance and grey indicates susceptibility. Abberiviations: doxyxycline (D), trimethoprim / sulfamethoxzole (SXT), clindamycin (CC) ciprofloxacin (CIP), erythromycin (E) and fusidicacid (FA). PVL: black indicates that Panton-Valentine leukocidin genes were detected, SCC*mec*, staphylococcal cassette chromosome *mec*elements; MLST, multilocus sequence.

DISCUSSIONS

The study indicated that prevalence of nasal MRSA colonization among healthy children in the eight primary schools in east Malaysia was 3.4% during the period from October 2012 to March 2013.In US for healthy children, the nasal colonization was ranged from 0.22% to 2.2% [24, 25].In Taiwan among children of ages 2 months to five years nasal colonization was 7.3% (26). Researchers in the United States reported that children with MRSA,SSTIs and no known exposure to healthcare environments presented with increasing frequency to the University Chicago Hospital between 1988 to 1989 and 1993 to 1995 [27].

A follow up study in 1998 to 1999 demonstrated a continued high rate of hospitalization for CA-MRSA disease at that institution [28]. As reported in several pediatric studies; however, and increasing trend in this regard has been reported in certain areas of United States recently. Creech*at al* reported that nasal MRSA colonization rate among healthy children in Nashville, TN,increased significantly from 0.8% in 2001 to 9.2% in 2004[13]. In the United States between 1998 and 2002 researchers reported a MRSA prevalence of 1.3% [29].

In another study in Taiwan MRSA carriage was 7.8% among healthy children from 2005 to 2008, with rates ranging from 6.2% to 9.5% in different geographical areas [30]. Which was considered to be higher than Taiwanese adult populations surveyed during the same period (3.8%; *P*, 0.0001), (30).The CA-MRSA isolates were initially identified in pediatric populations and subsequently reported in adult population [4].In a prospective observational study in 812 US Army soldiers 3% were colonized with CA-MRSA and 9 of whom (38%) developed soft-tissue infections during the study period (12). In this study the results are comparable to other studies .SSTIs infections remained the most common manifestations of CA-MRSA, severe diseases such as necrotizing pneumonitis ,necrotizing fasciititis osteomyelitis, pyomyositis, septic embolism, and venous thrombosis were not uncommon and previously causeddeath in healthy children (31,6). The causes of the increasing incidence of CA-MRSA infection in previously healthy hosts are not completely understood, and the factors influencing CA-MRSA virulence remain an issue of ongoing debate (32,9).

Evidence further suggest that compared to methicillin-susceptible *S.aureus* (MSSA), colonization with MRSA imposes a significantly greater risk for development of subsequent infections [32]. In the past few years, the prevalence of MRSA colonization increased significantly higher among healthy hosts during CA-MRSA epidemics (13). The carriage of MRSA genotypes was significantly higher among children than adults [15].

Furthermore, living with young children was associated with increased risk of MRSA colonization in adults [31]. When combined, these observations strongly suggested young children were the major reservoir of MRSA in the community and were the main population responsible for the accelerated transmission of CA-MRSA (29).

Molecular characterization in the present study identified three clones from the 17 available MRSA isolates. The clone of ST30-SCCmec IV-PVL-positive, known as the southwest Pacific clone, is a common CA-MRSA clone,

which prevailed in Australia, Singapore, Hong Kong, the Philippines, Japan and Malaysia [35-39]. This clone was also the most common clone identified in the present study and accounted for about 60% of the isolates.

The clone of ST45-SCC*mec* IV-PVL-negative, accounting for about 30% of the isolates in the present study, is a healthcare associated (HA)-MRSA clone, which prevailed in Europe [40] and recently in Hong Kong [41,42].This clone (ST45-SCC*mec* IV/V) was associated with the increasing isolation of multi susceptible-MRSA in five Hong Kong hospitals from 1995 to 2005 [41] and also accounted for seven of 28 MRSA isolates from healthy children aged 2-5 years attending day care centers and kindergartens in Hong Kong during 2009-2010 [42]. The third clone identified in the current study, characterized as ST239-spa t037-SCCmec III-PVL-negative, is an epidemic HA-MRSA clone in Asia and worldwide [36, 37]. The identification of HA-MRSA isolates from the healthy children without risk factors (?) suggests the penetration of HA-MRSA isolates into the community.

There were several limitations in our study: 1) our survey estimated only nasal colonization not the colonization of other body sites that underestimated the true prevalence of *S. aureus.* 2).No sample was taken from the household.3) Information on family overcrowding was not available. 4) Subjects were recruited from eight schools; therefore the prevalence of MRSA and MSSA may not reflect the entire population of Sabah, Malaysia.

CONCLUSIONS

3.4 % of healthy children in three districts of Sabah were colonized by MRSA in the nares during the study period from October 2012 to march 2013.MRSA nasal carriage in the children may spread in the community. Three main CA- MRSA clones were identified and seem to have spread in the three districts .Future researchers have to determine several factors of nasal colonization and the efforts must be made to detect and prevent the spread of CA-MRSA in the population.

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Conflictof Interest: Authors have no conflict of interests.

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